HOSPITAL DE CLINICAS
Dr. Manuel Quintela

CITOMETRÍA DE FLUJO EN LAS GAMAPATÍAS MONOCLONALES

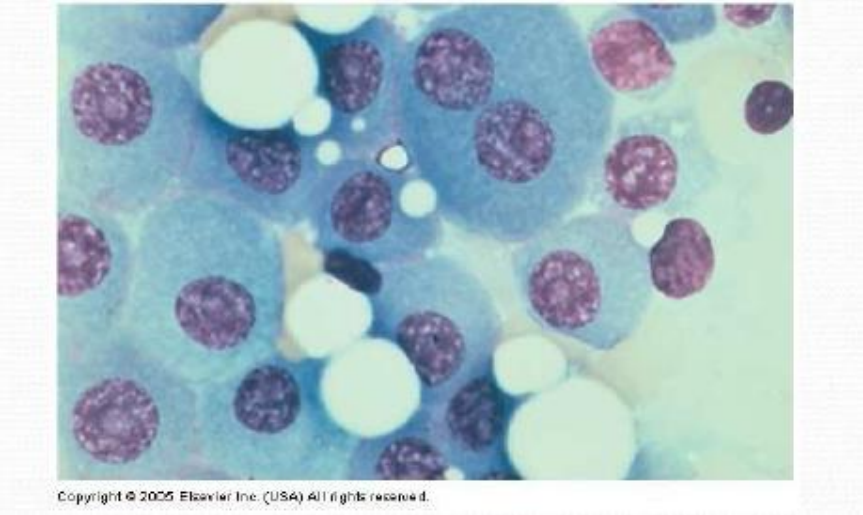
Dra. Daniela Lens

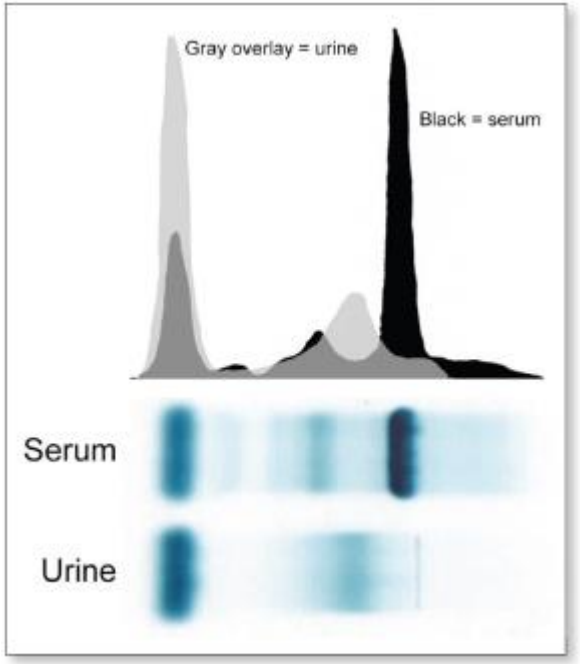
Laboratorio de Citometría y Biología Molecular.

Hospital de Clínicas. Universidad de la República.

Definición

- Resultan de la expansión de una clona de plasmocitos o linfocitos B que producen una Ig monoclonal o un fragmento de ella que puede detectarse en sangre y/o orina

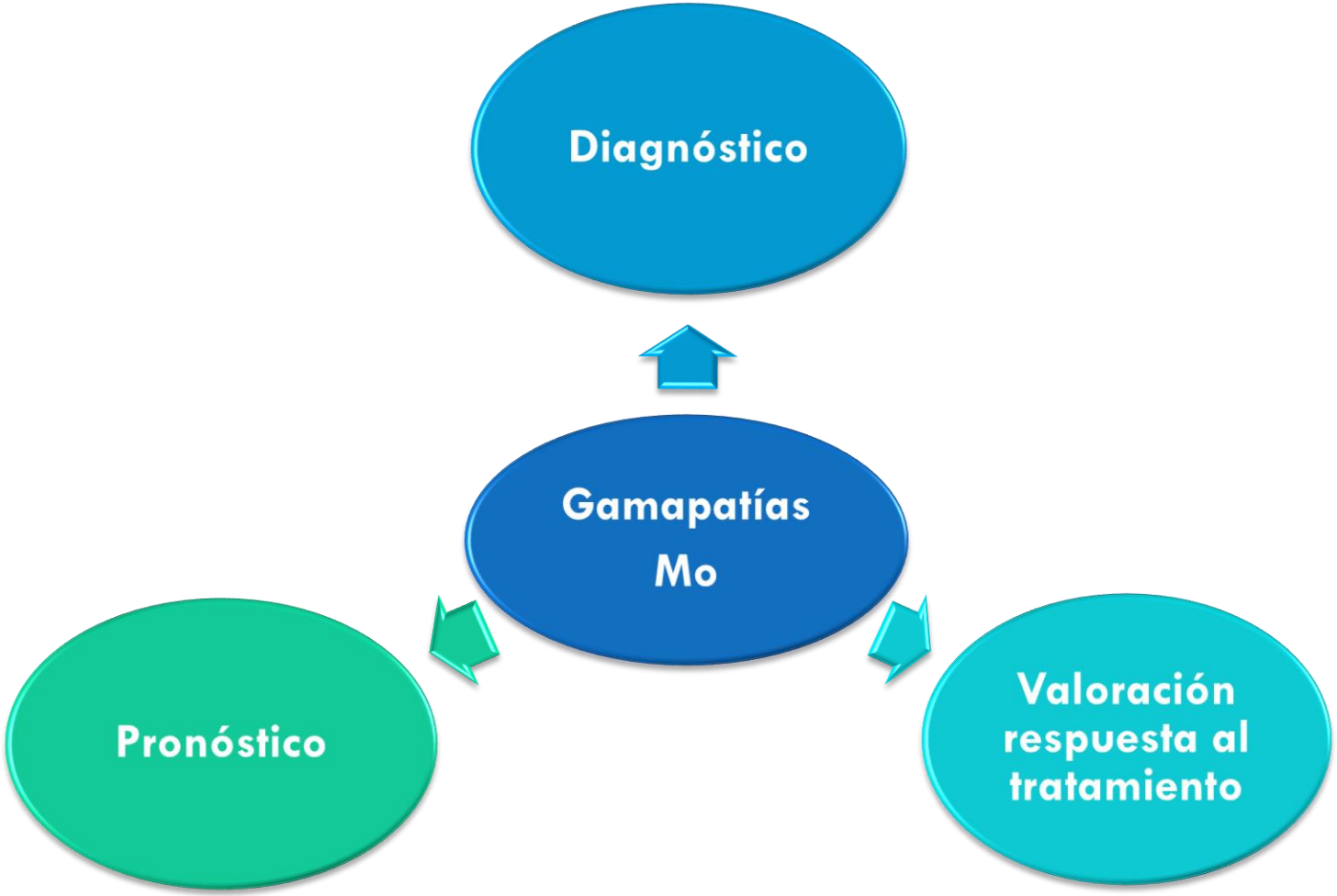


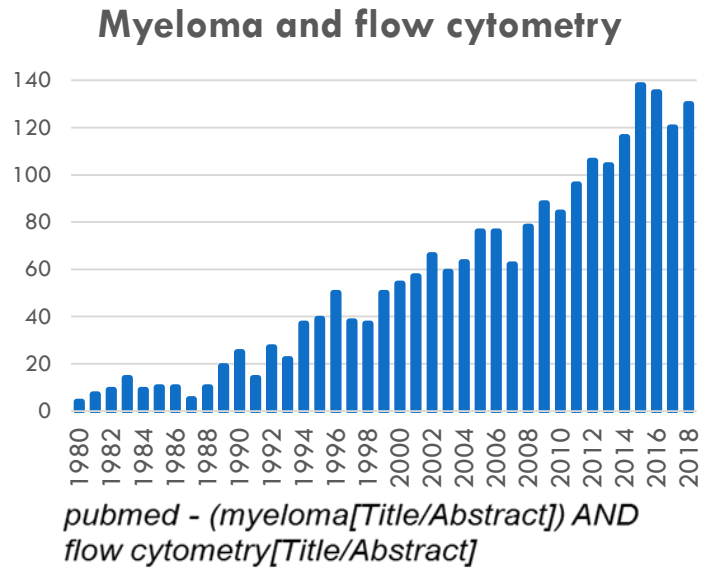


Gamapatías Monoclonales

- Gamapatía Monoclonal de Significado Incierto
- Mieloma Múltiple
- Enfermedad de Waldestrom
- Enfermedad de cadenas pesadas
- Amiloidosis primaria
- Plasmocitoma solitario

CF en Gamapatías monoclonales





Caracterización inmunofenotípica de las CP

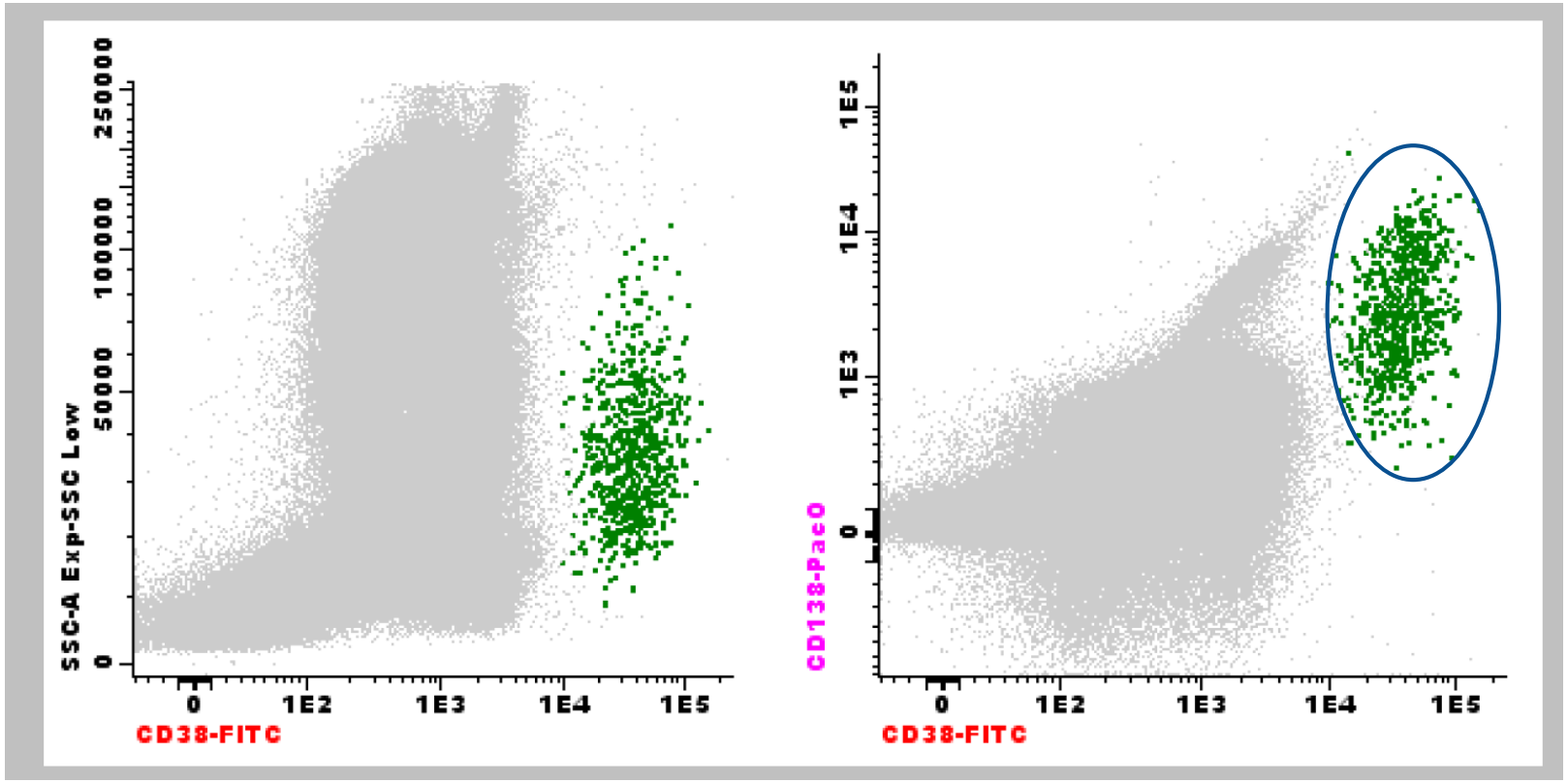
Identificación de la CP

- CD38/CD138

Caracterización de la CP

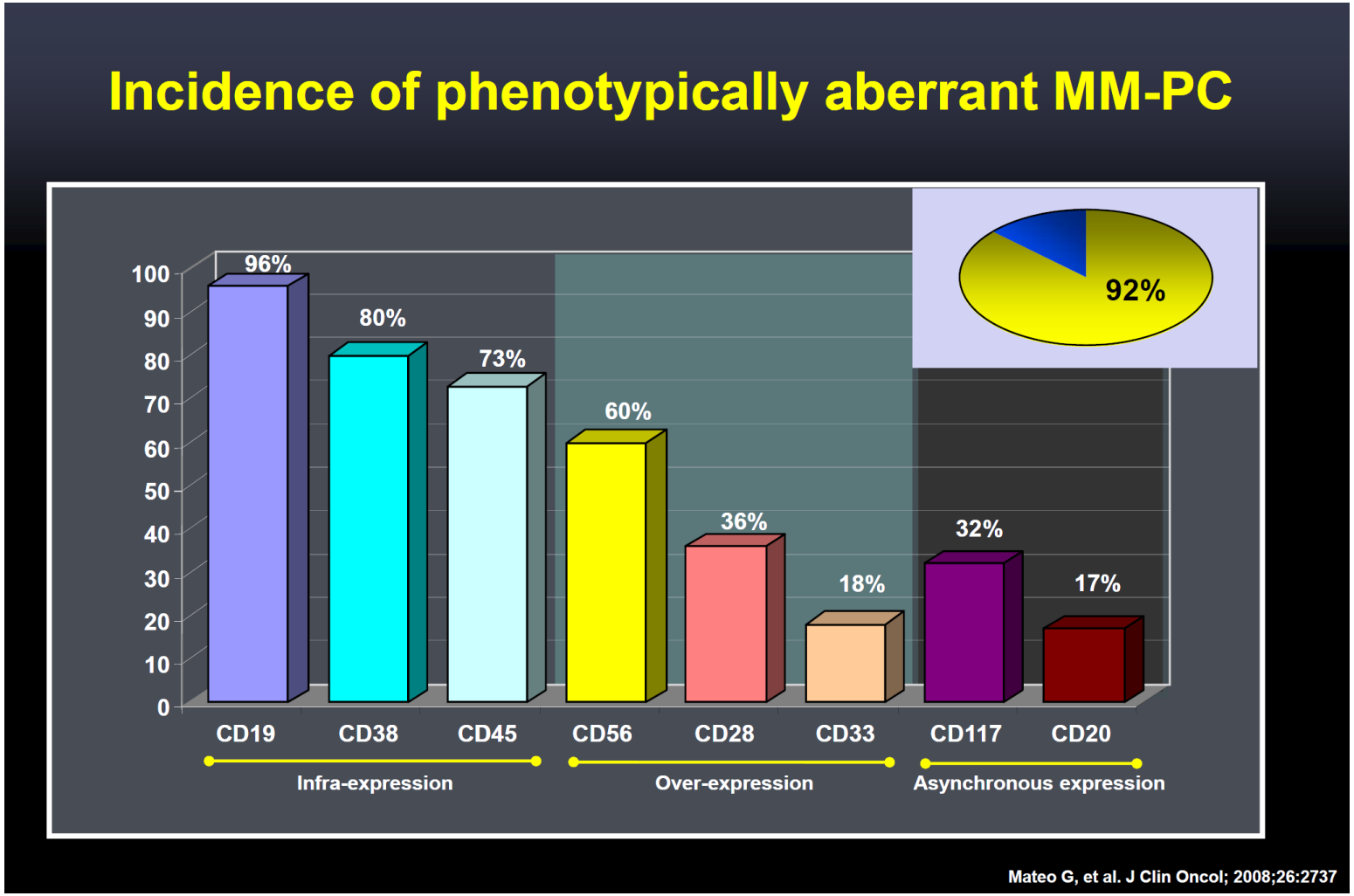
- Discriminación CP normal / CP patológica:
  - Clonalidad
  - Marcadores aberrantes

CF en discrasias plasmocitarias



Identificación por CD138/CD38

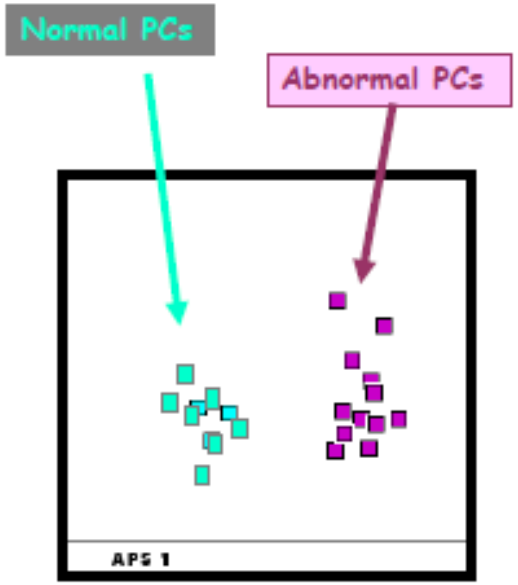
Patrones aberrantes en MM



Panel Euroflow para Ga.Mo

Tube	PacB	PacO	FITC	PE	PerCP Cy5.5	PECy7	APC	APCH7
1	CD45	CD138	CD38	CD56	β2micro	CD19	CyIgκ	CyIgλ
2	CD45	CD138	CD38	CD28	CD27	CD19	CD117	CD81

Abnormal PC detection /classification in MGUS & MM (APS view)



Most informative markers	
CD19·PE Cy7-A LOGICAL	12.16
CD45·PacB-A LOGICAL	11.81

EuroFlow

Responsible scientist· J.Flores

Utilidad de la CF en pronóstico de Ga.Mo

Predicción de evolución Ga.Mo	• % CP anormales / CP totales
Marcadores pronósticos en MM	• Expresión de marcadores específicos (aberrantes) CD45/CD56/CD117/CD28/CD81
Detección de EMR en MM	• Cuantificación de CP anormales

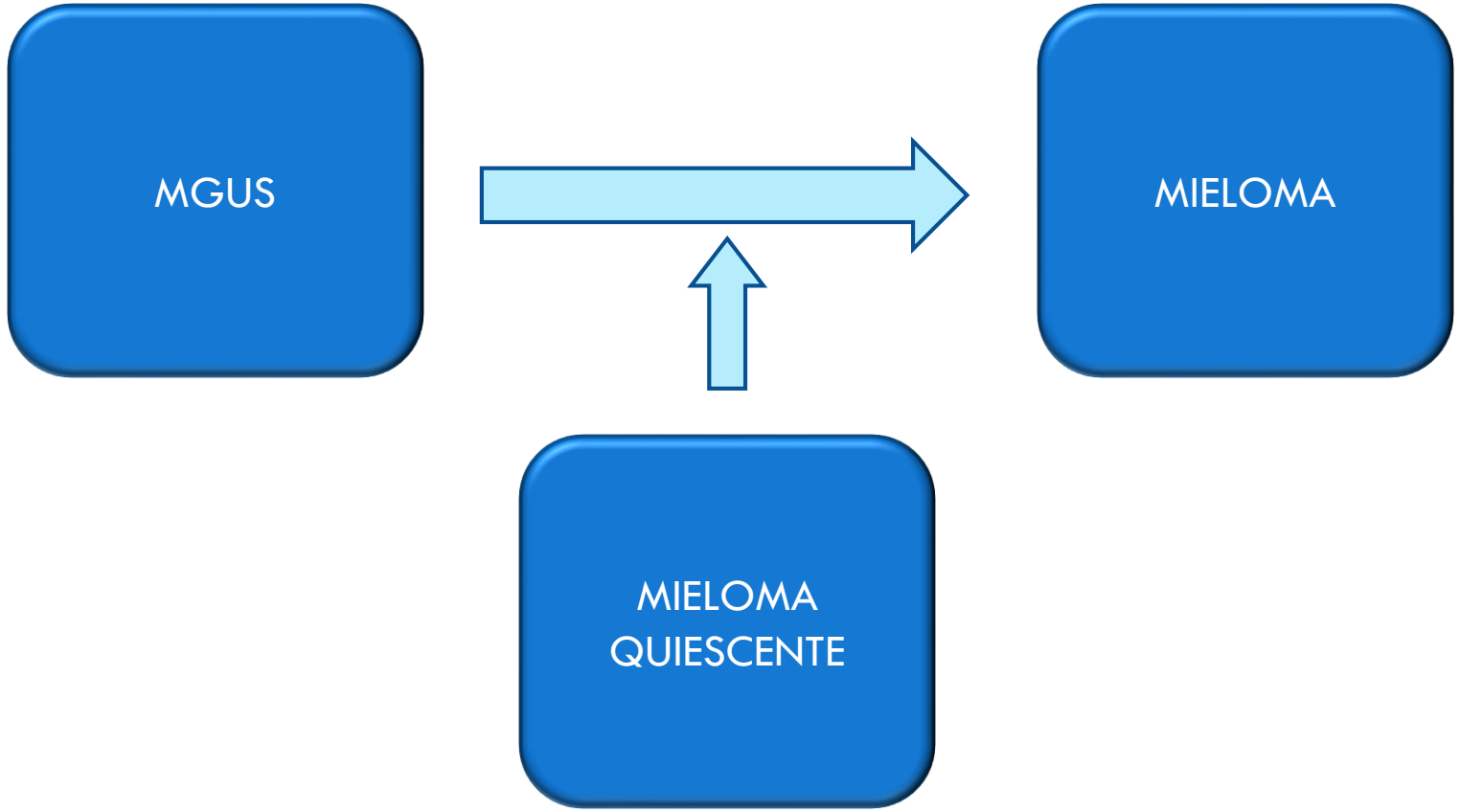
Rawstron et al. Haematologica 2008;93:431-438

Utilidad de la CF en pronóstico de Ga.Mo

Predicción de evolución MGUS o SM a MM

- % CP anormal /CP Totales

Transformación MGUS o SM a MM



RIESGO DE TRANSFORMACIÓN:
1% /año MGUS a MM
10% / año SM a MM

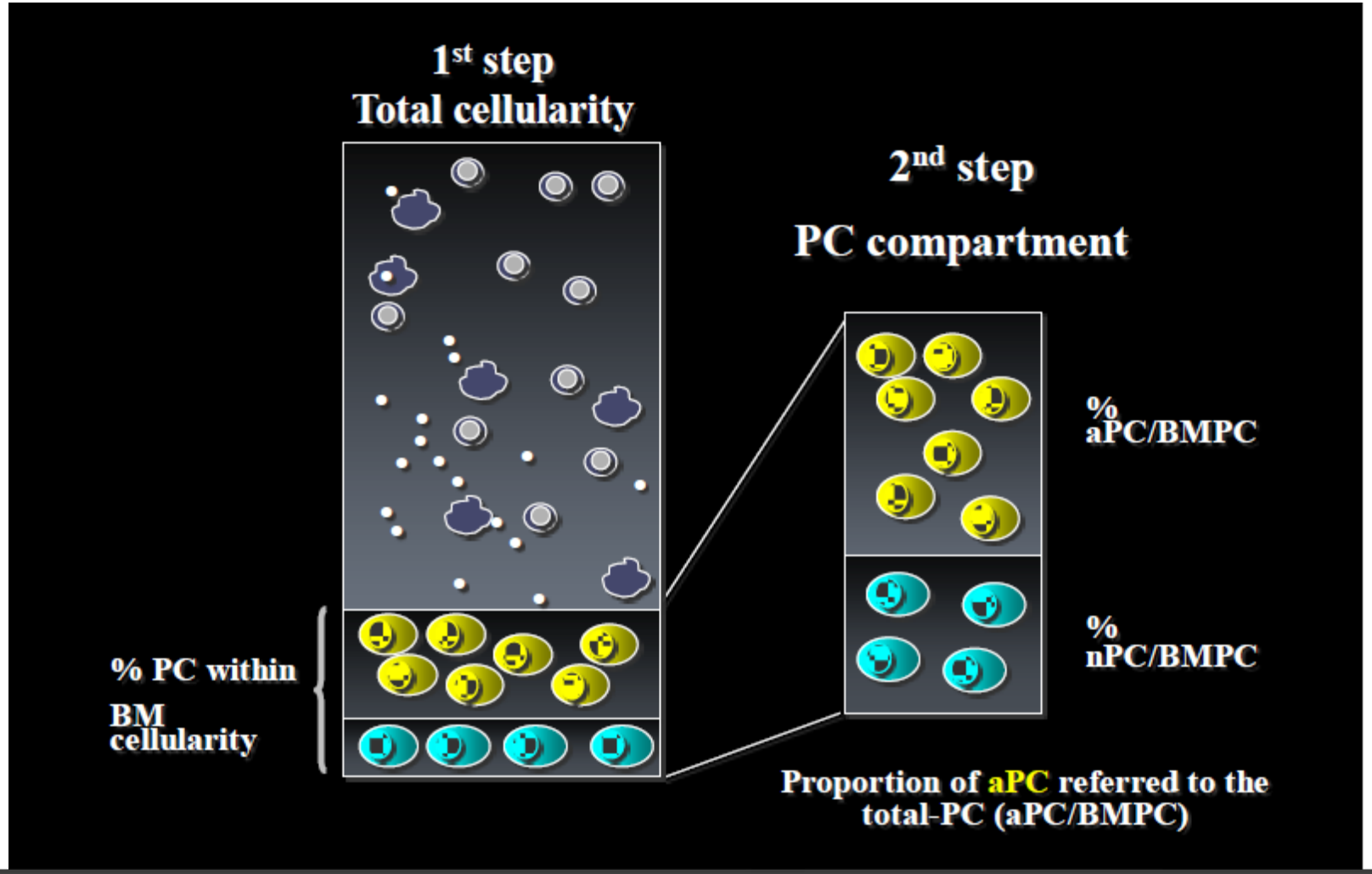
Criterios Diagnósticos

MGUS	MM smoldering	MM (sintomático)
▪ Componente M < 3 g/dl	▪ Componente M≥ 3 g/dL (suero) or ≥ 500 mg/24 hrs (orina)	▪ Gamapatía monoclonal
▪ CP clonales en MO < 10%	▪ CP clonales en MO 10% - 60%	+ (1 o más):
▪ Sin eventos que definan MM	▪ Sin eventos que definan MM	▪ ≥ 1 CRAB*
		▪ CP clonales en MO 10% - 60%
		▪ Ratio de cadenas livianas ≥ 100
		▪ > 1 lesion focal en RNM

*C: Hipercalcemia
R: Insuficiencia renal
A: Anemia
B: Enfermedad ósea

Rajkumar SV, et al. Lancet Oncol. 2014;15:e538-e548.

Análisis de las CP en MO por CF



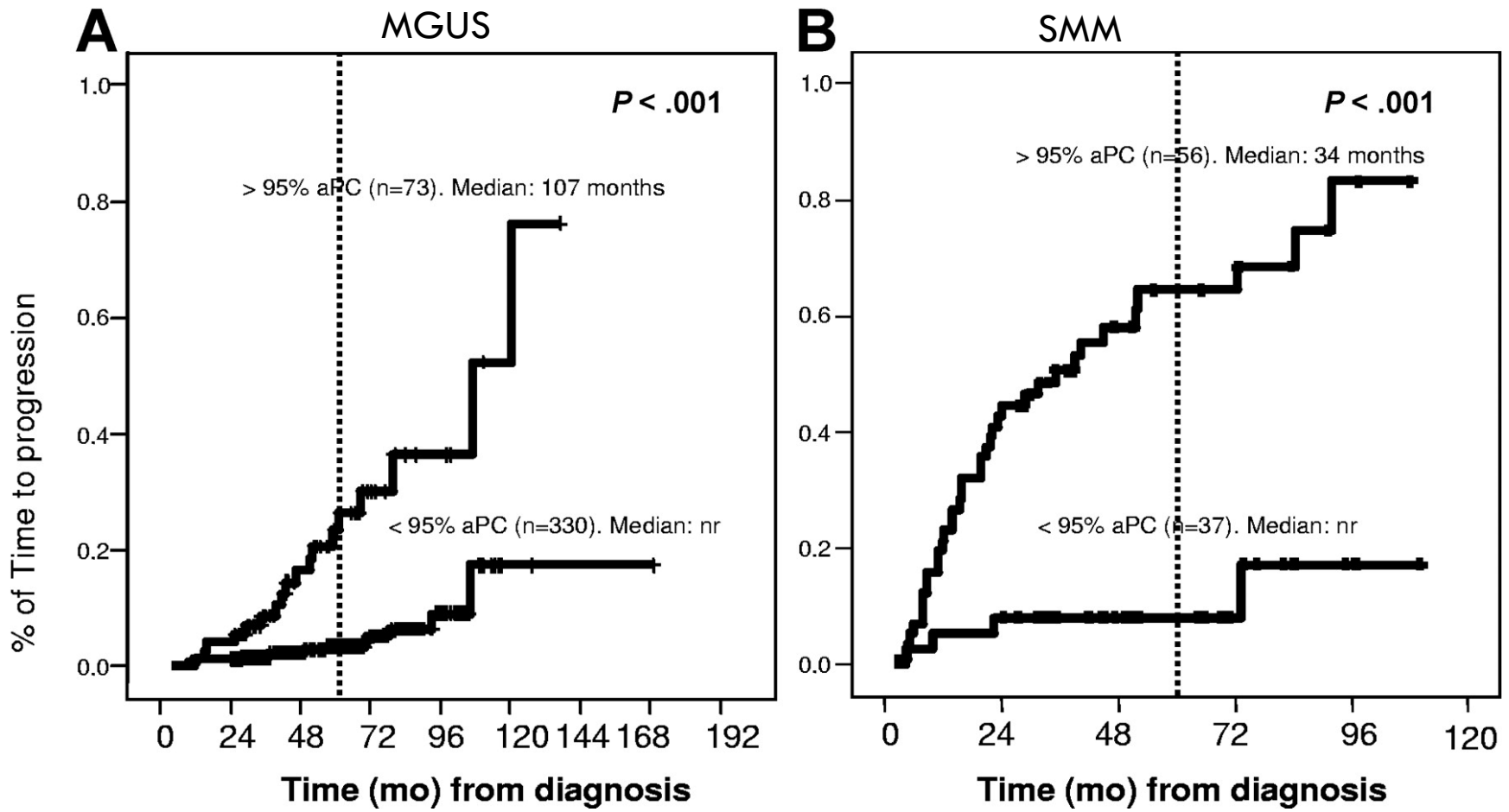
de Orfao y col.

Diferenciación MGUS - MM

% of cases	MGUS	SMM	MM
>5% N-PC/BMPC	82%	40%	14%
≤5% N-PC/BMPC	18%	60%	86%

Perez Persona et al Blood 2007
Paiva et al Blood 2009

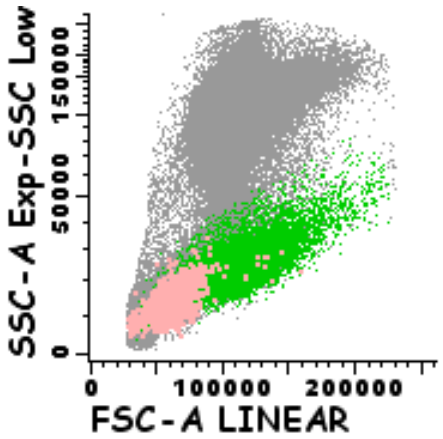
Riesgo de transformación en MGUS y SMM

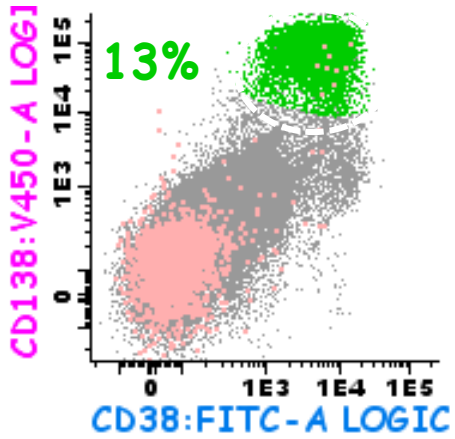


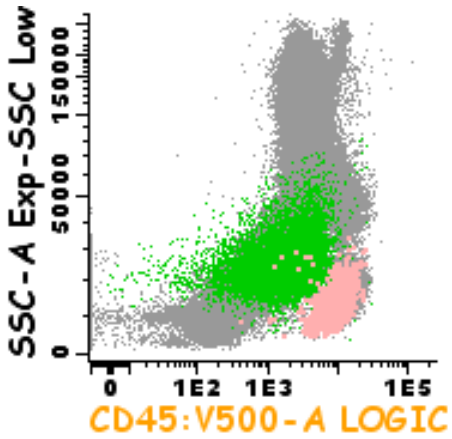
Perez-Persona et al. Blood 2007

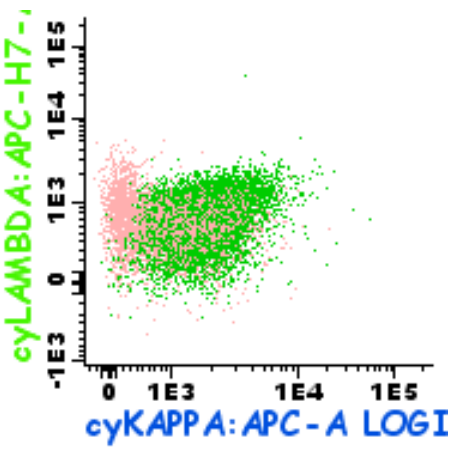
ANÁLISIS DE CASOS

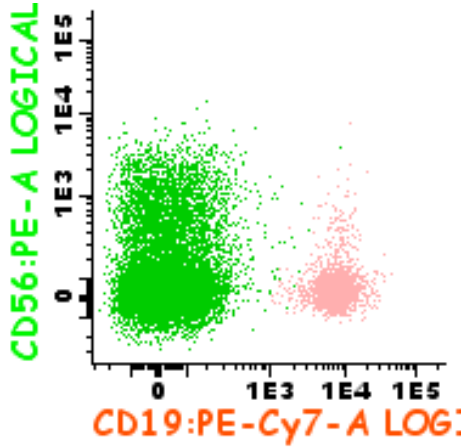
tubo	V450	V500	FITC	PE	PerCP Cy5.5	PECy7	APC	APCH7
PCD1	**CD45**	**CD138**	CD38	CD56		**CD19**	CyIgK	CyIgL
PCD2	**CD45**	**CD138**	CD38	CD28	CD27	**CD19**	CD117	CD81

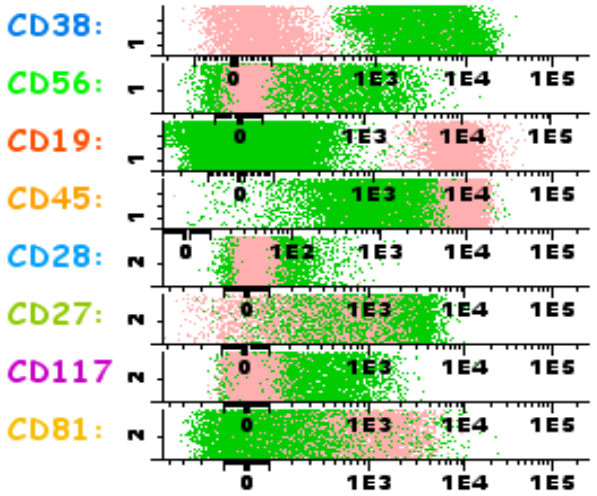












tubo	V450	V500	FITC	PE	PerCP Cy5.5	PECy7	APC	APCH7
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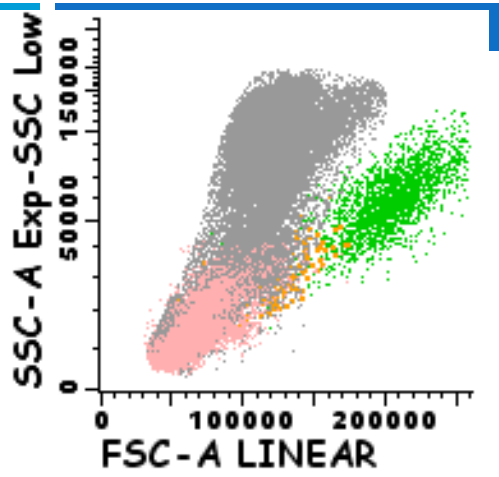
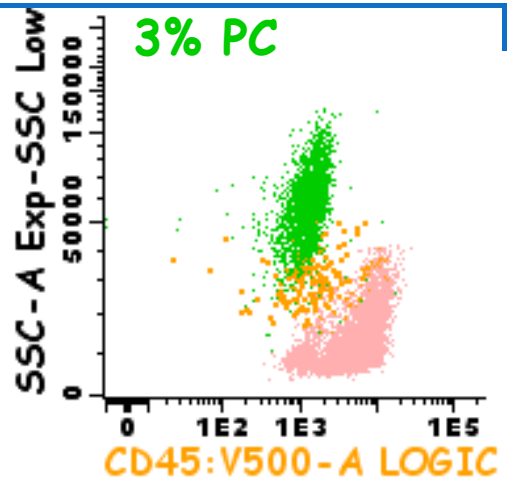
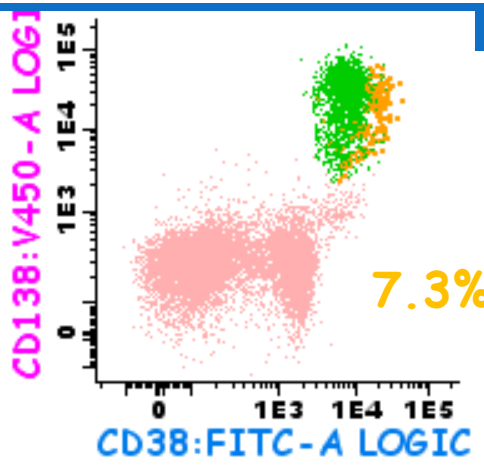
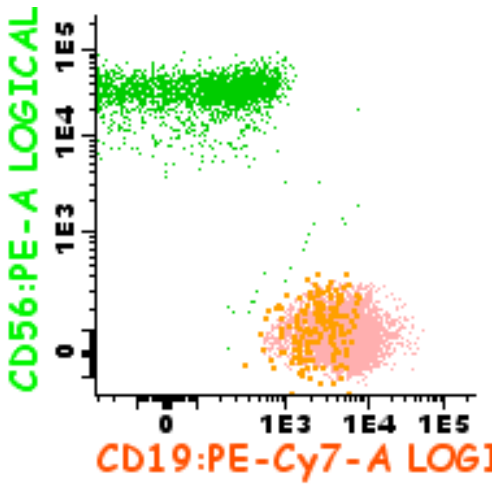
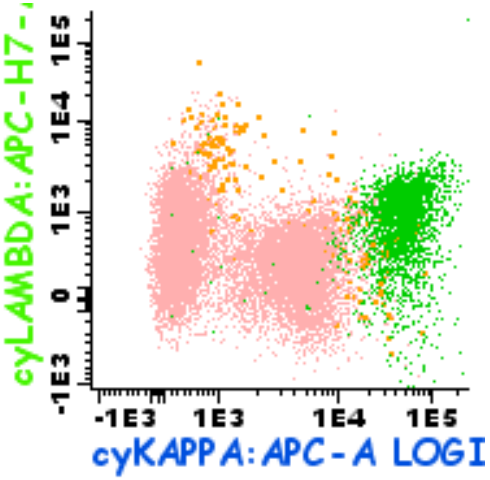






7.3% PC normales





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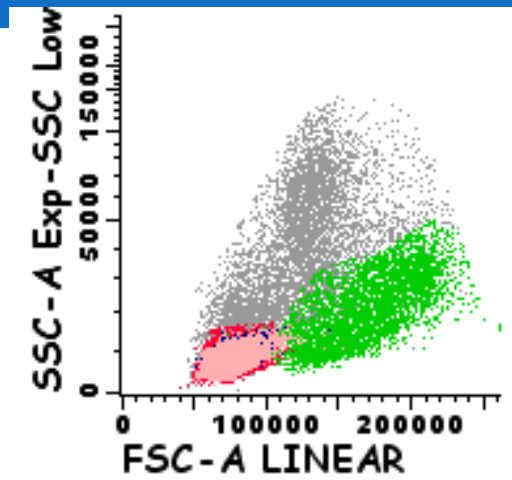

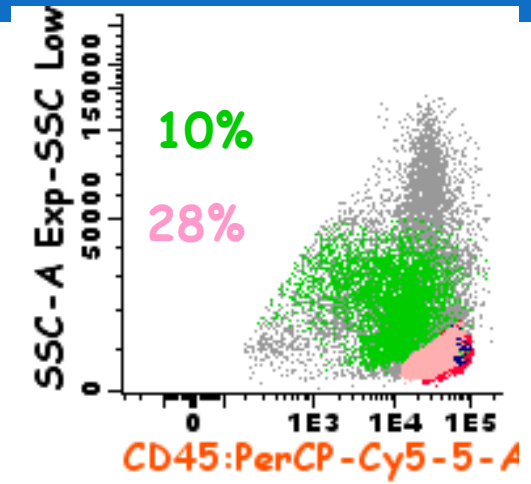

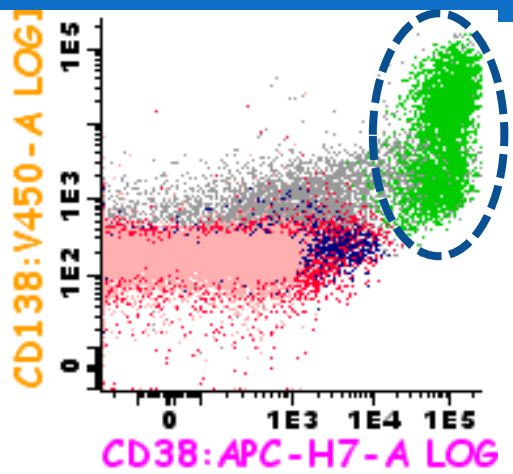

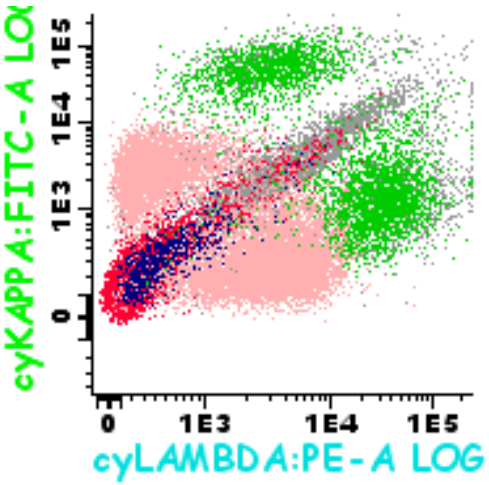

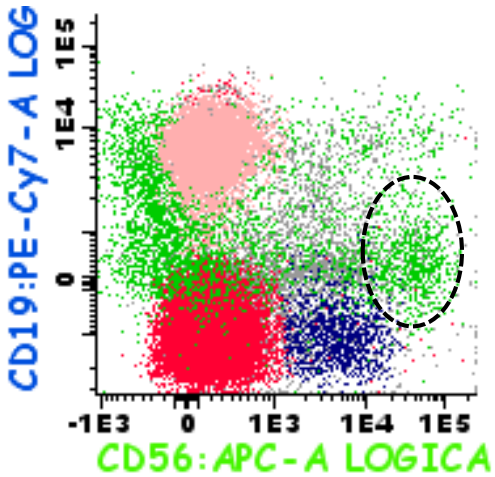

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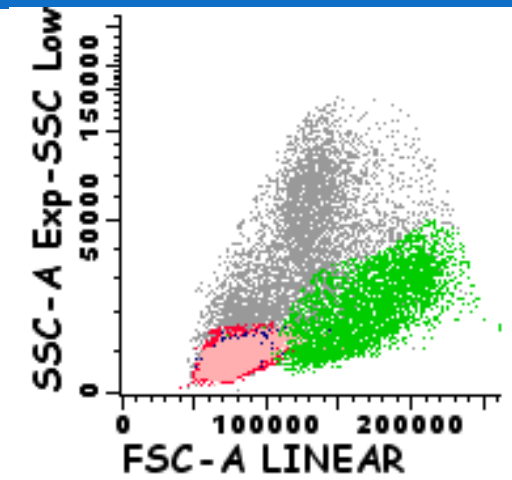
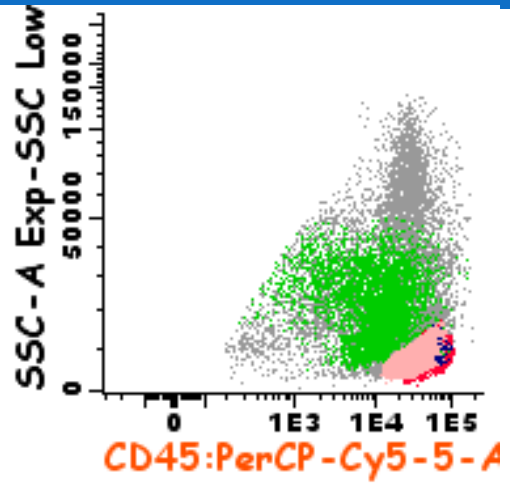
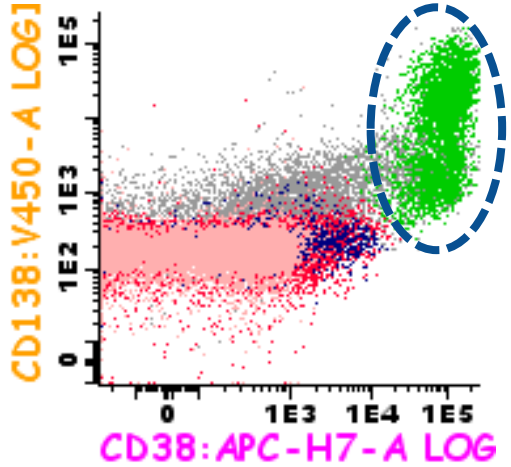
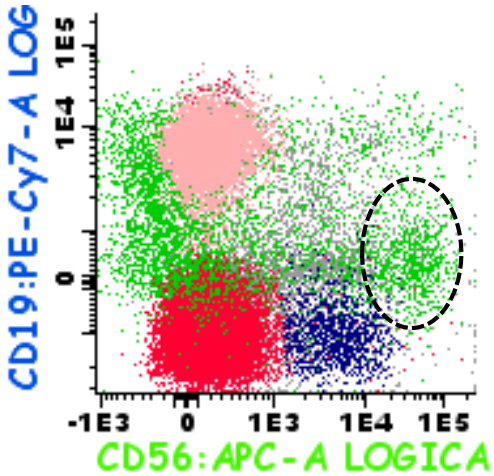
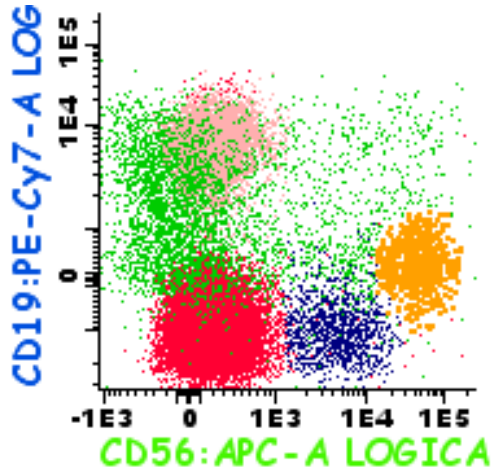
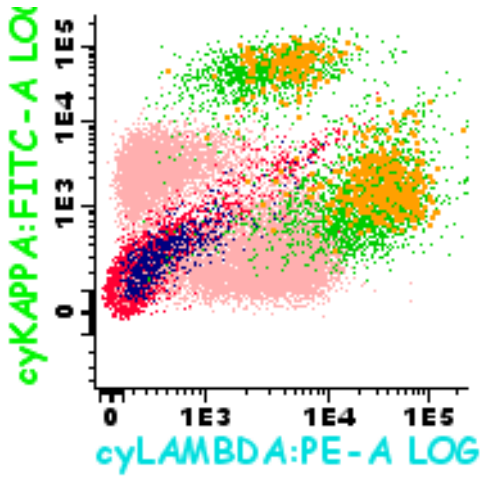












EMR por CF en MM

Detección de EMR en MM

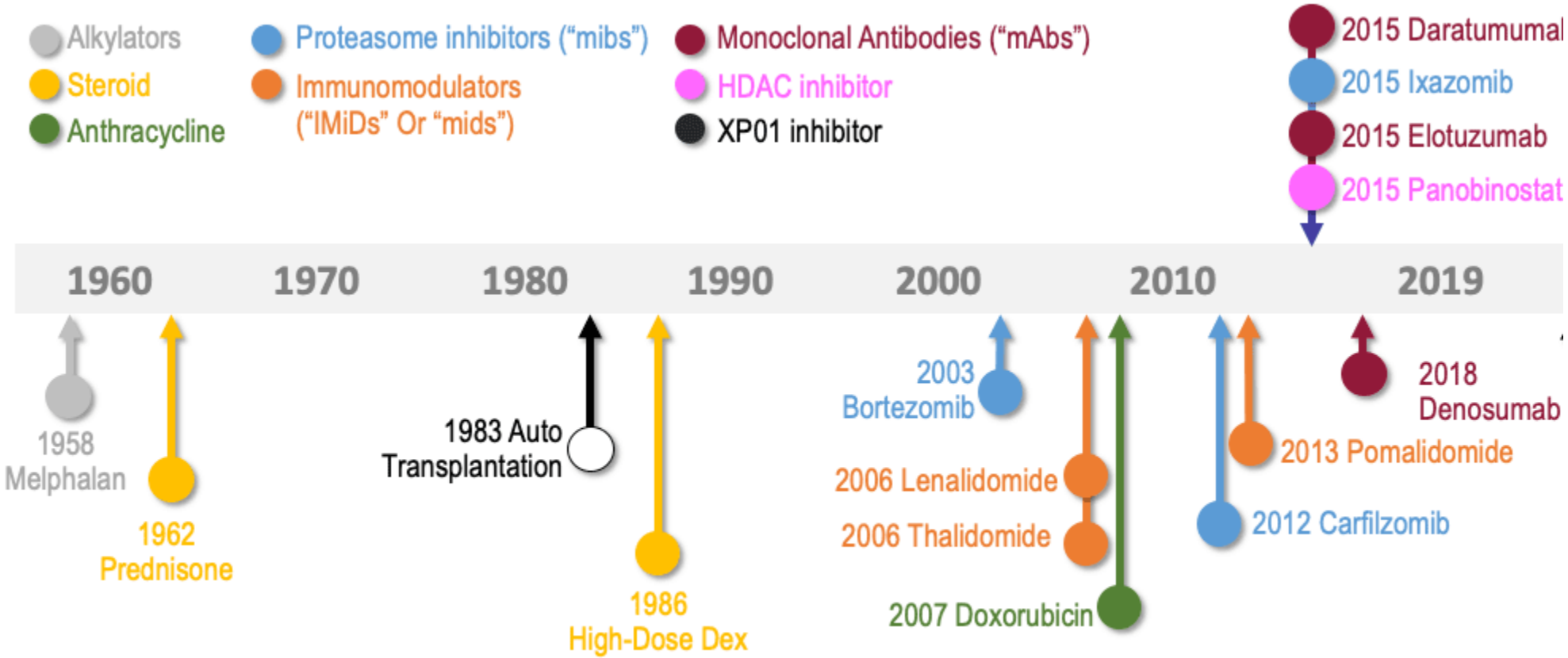
- Cuantificación de CP anormales

Tratamiento en el MM

- En la última década el tratamiento del MM ha incorporado un gran número de agentes terapeúticos

- Se ha mejorado considerablemente el número de respuestas completas y los índices de sobrevida

Cronología temporal de la terapéutica en el MM



Auto = autologous; Dex= dexamethasone.

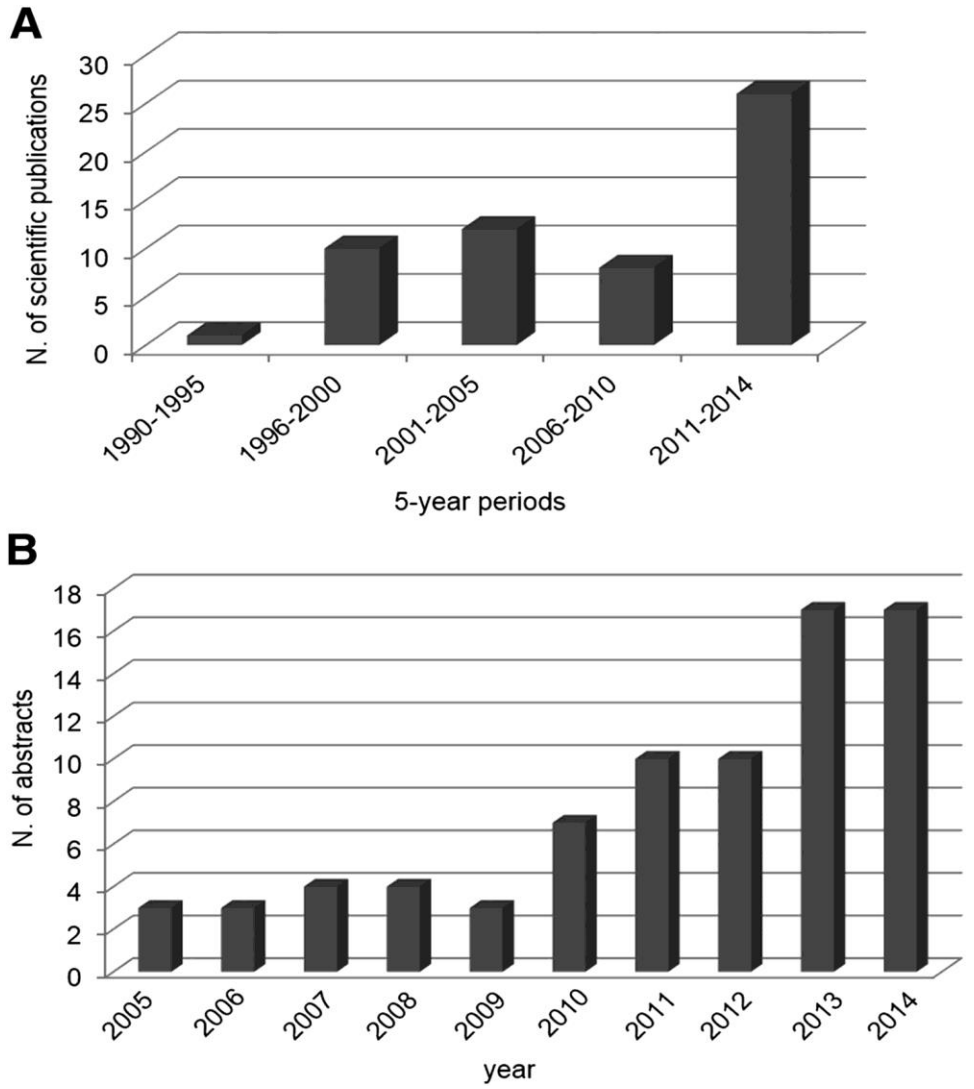
Tariman, J. *Nurs Clin North Am.* 2017;52(1):65-81. DRUGS@FDA.gov

Tratamiento en el MM

- Sin embargo un porcentaje considerable de pacientes recaen
- En varios ensayos clínicos, la RC no logra separar los grupos de pacientes que recaen de los que no recaen
- Se requieren métodos mas sensibles para la valoración de la respuesta

Enfermedad mínima residual en MM

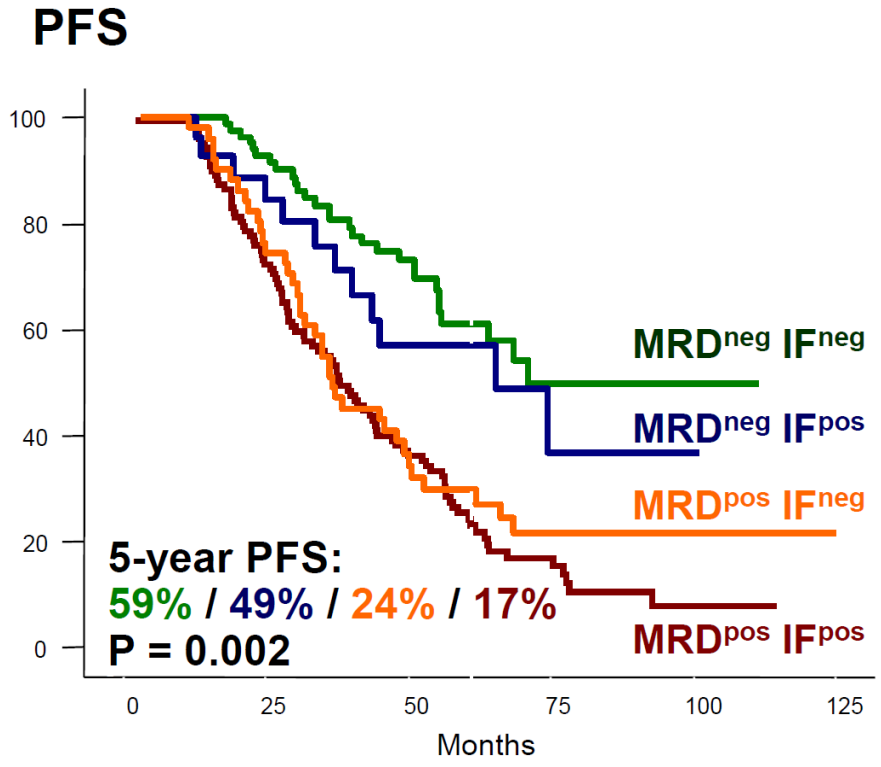
N° de publicaciones en PUBMED y N° de abstracts en ASH



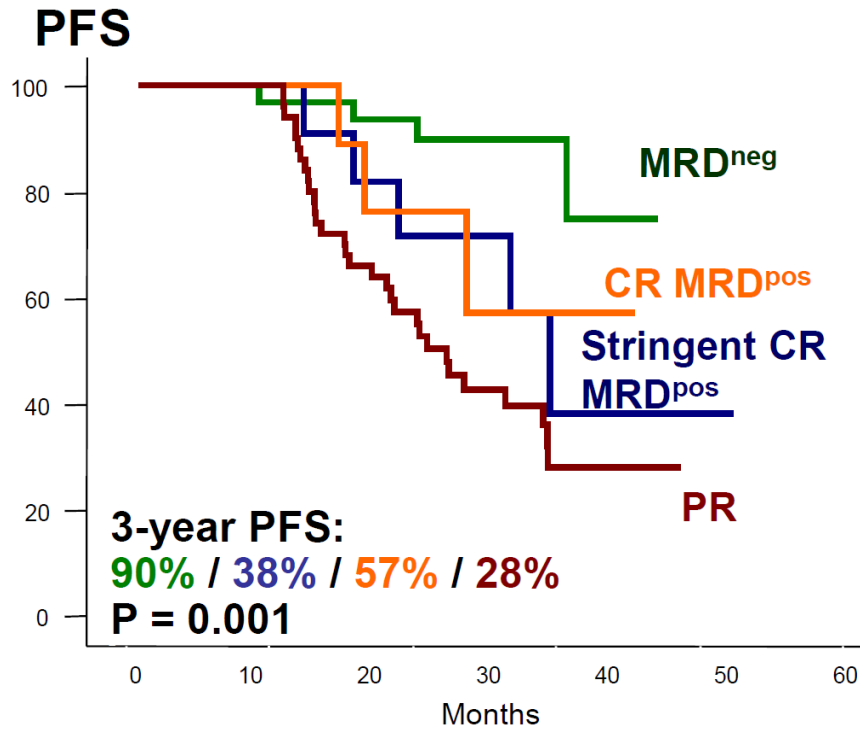
Bruno Paiva et al. Blood 2015;125:3059-3068

blood

CF vs. inmunofijación como predictor de respuesta

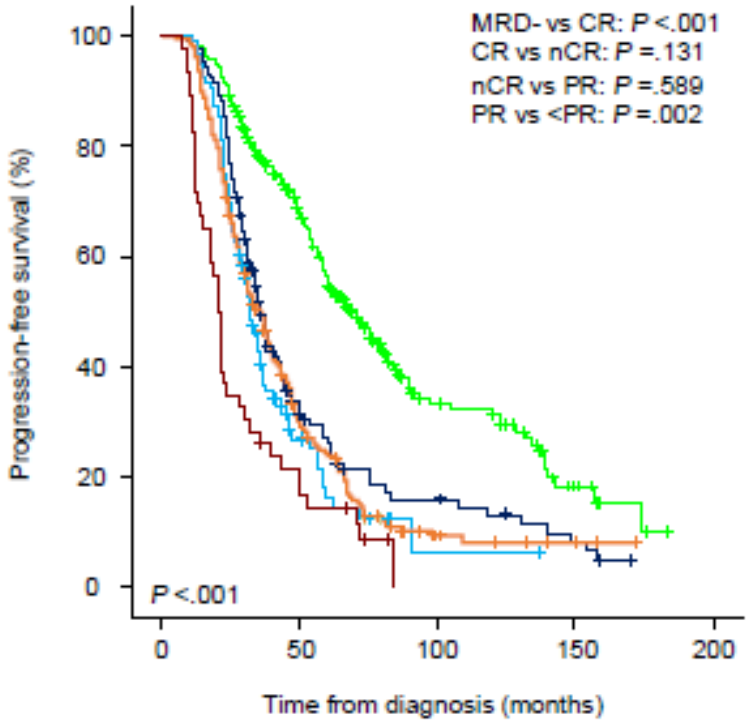


Pavia et al (2008) Blood 112: 4017
GEM2000: VBMCP/VBAD ➔ HDM ASCT

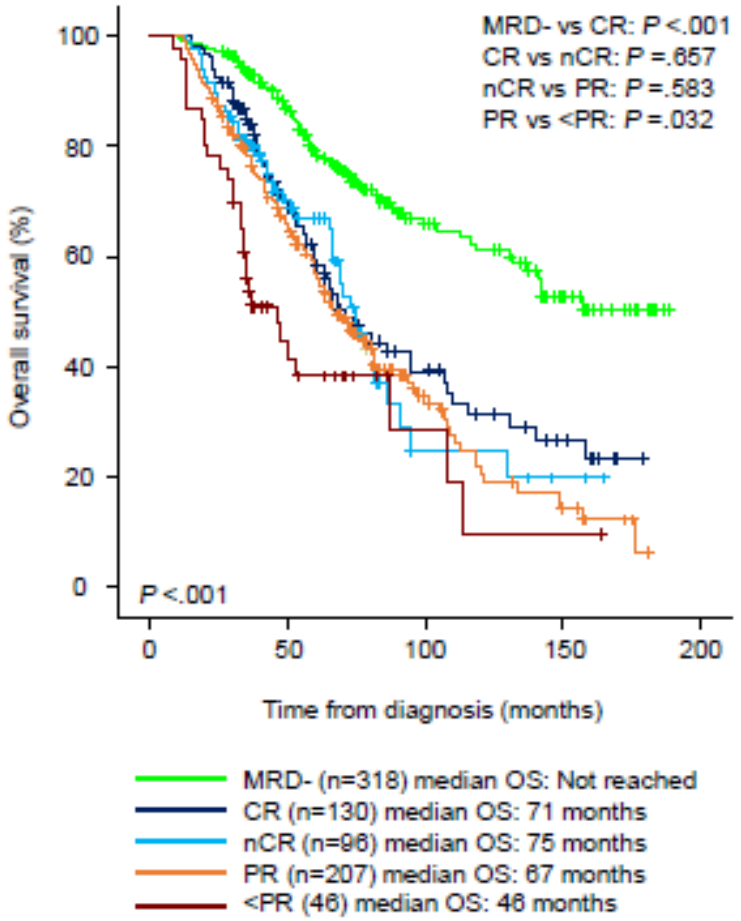


Pavia et al (2011)
J Clin Oncol 29(12): 1637-GEM2005 >65y

…But the true value of CR relies in the MRD status, and CR w/o MRD is no better than PR



GEM2000, GEM2005MENOS65, GEM2005MAS65, GEM2010MAS65



Orfao y col.
ESCCA 2016

Técnicas para estudio de EMR (2014)

	Multidimensional (≥8-color) FC	Molecular ASO-PCR	High-throughput sequencing	PET-CT
Diagnostic sample	Important but not mandatory	Mandatory	Mandatory	?
Time	2-hours	5 days	7 days	2-hours
Availability	High	Intermediate	?	Intermediate
Cost	~150 euros	~450 euros	??	High
Standardization	Ongoing	Yes (Biomed)	Yes (Sequenta)?	?
Sensitivity	10^{-5}	10^{-5} – 10^{-6}	10^{-6}	High (?)

NEXT GENERATION FLOW (NGF):
EuroFlow-IMF NGF-MRD MM Approach

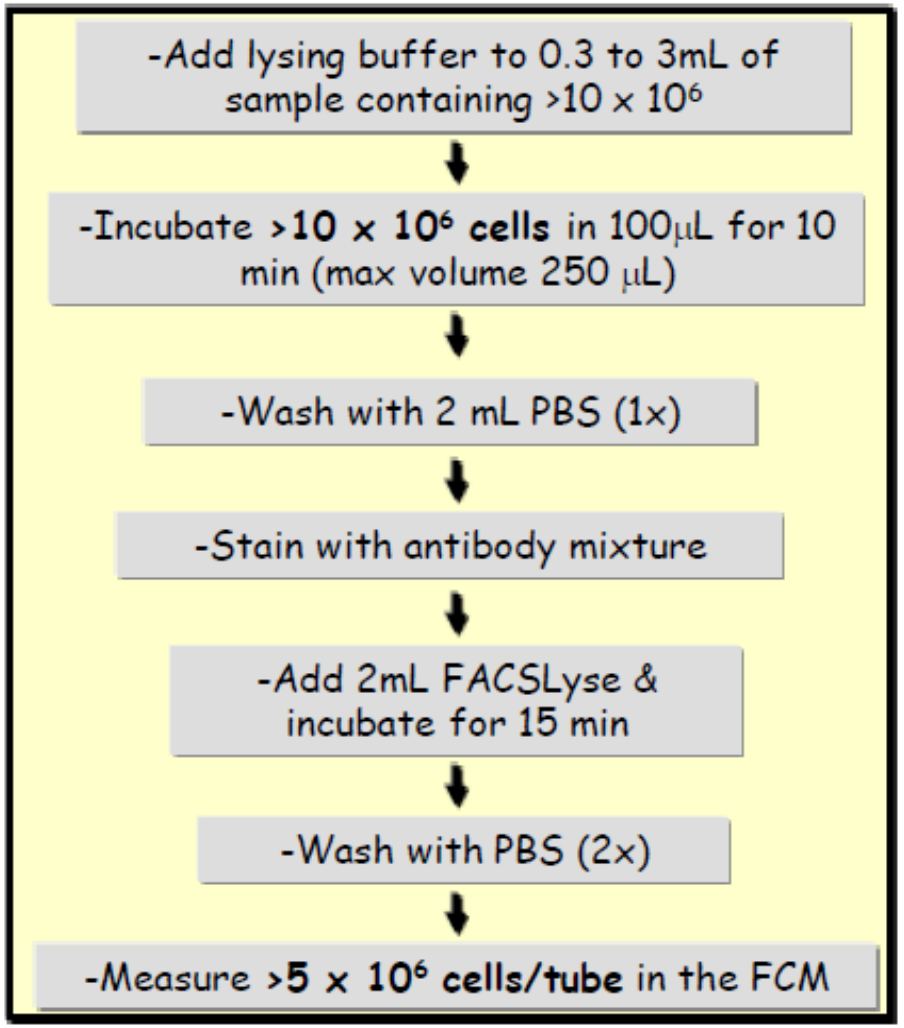
Tube	BV421	BV510	FITC	PE	PerCP Cy5.5	PECy7	APC	APCC750
1	CD138	CD27	CD38	CD56	CD45	CD19	CD117	CD81
2							cyKappa	cyLambda

Standardized sample processing → systematic acquisition of ≥ 10^7

Infinicyt software (version 1.8.0 RC6): PCA, file merge, automated gating

Procesamiento de la muestra en NGF

New Sample Preparation SOP


-Add lysing buffer to 0.3 to 3mL of sample containing >10 x 10⁶
-Incubate >10 x 10⁶ cells in 100µL for 10 min (max volume 250 µL)
-Wash with 2 mL PBS (1x)
-Stain with antibody mixture
-Add 2mL FACSLyse & incubate for 15 min
-Wash with PBS (2x)
-Measure >5 x 10⁶ cells/tube in the FCM

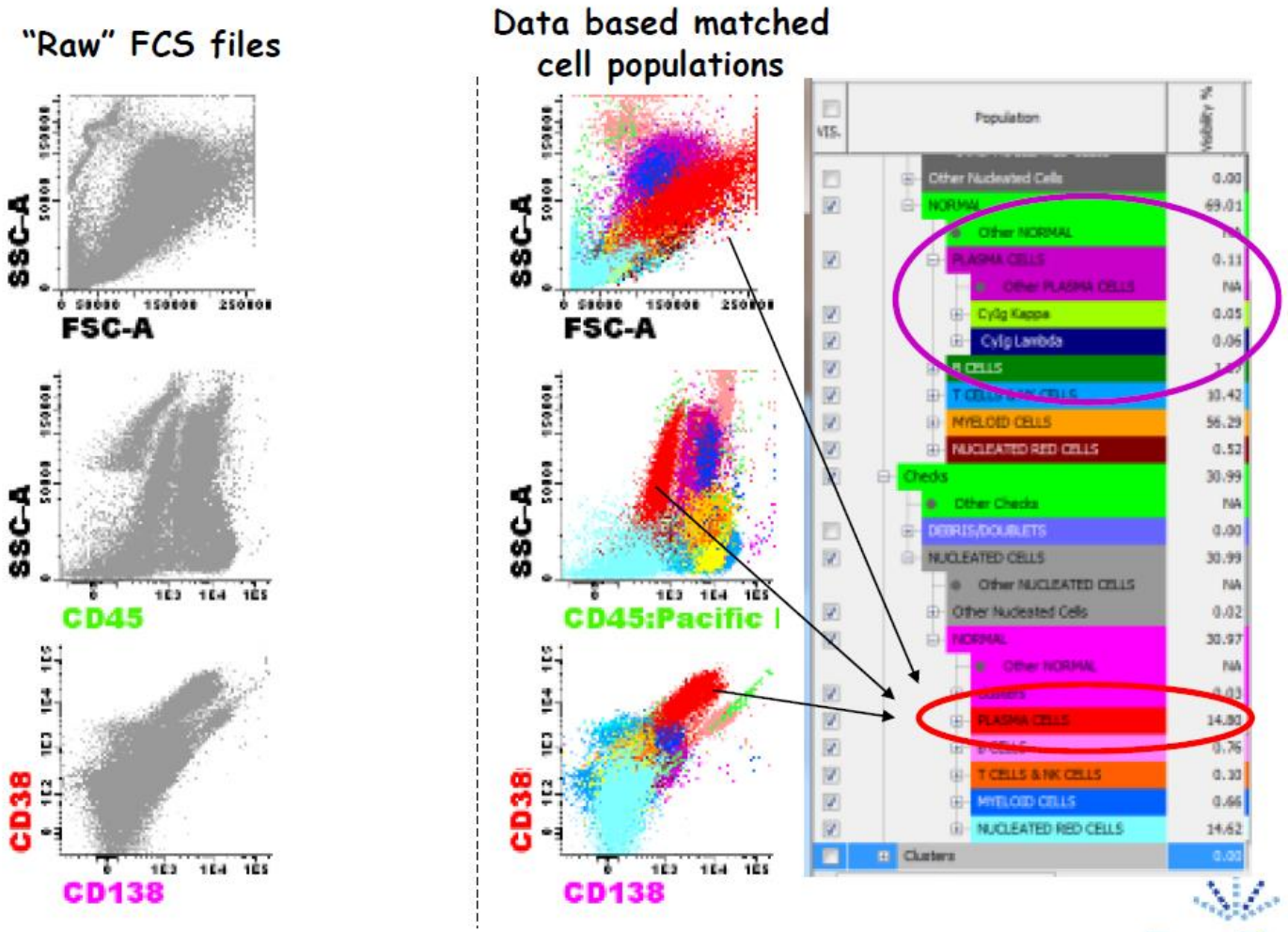
Reporte EMR

- Se debe concluir EMR negativa o positive

- Se debe incluir LOD y LOQ

- LOD= 20 / N° cel nucleadas adquiridas x100
- LOQ = 40 / N° cel nucleadas adquiridas x100

Análisis automático



CPN
CPC

Mastocitos
Eritroblastos
Progenitores B

International Myeloma Working Group consensus criteria for response and minimal residual disease assessment in multiple myeloma

Shaji Kumar, Bruno Paiva, Kenneth C Anderson, Brian Durie, Ola Landgren, Philippe Moreau, Nikhil Munshi, Sagar Lonial, Joan Bladé, Maria-Victoria Mateos, Meletios Dimopoulos, Efstathios Kastritis, Mario Boccadoro, Robert Orlowski, Hartmut Goldschmidt, Andrew Spencer, Jian Hou, Wee Joo Chng, Saad Z Usmani, Elena Zamagni, Kazuyuki Shimizu, Sundar Jagannath, Hans E Johnsen, Evangelos Terpos, Anthony Reiman, Robert A Kyle, Pieter Sonneveld, Paul G Richardson, Philip McCarthy, Heinz Ludwig, Wenming Chen, Michele Cavo, Jean-Luc Harousseau, Suzanne Lentzsch, Jens Hillengass, Antonio Palumbo, Alberto Orfao, S Vincent Rajkumar, Jesus San Miguel, Herve Avet-Loiseau

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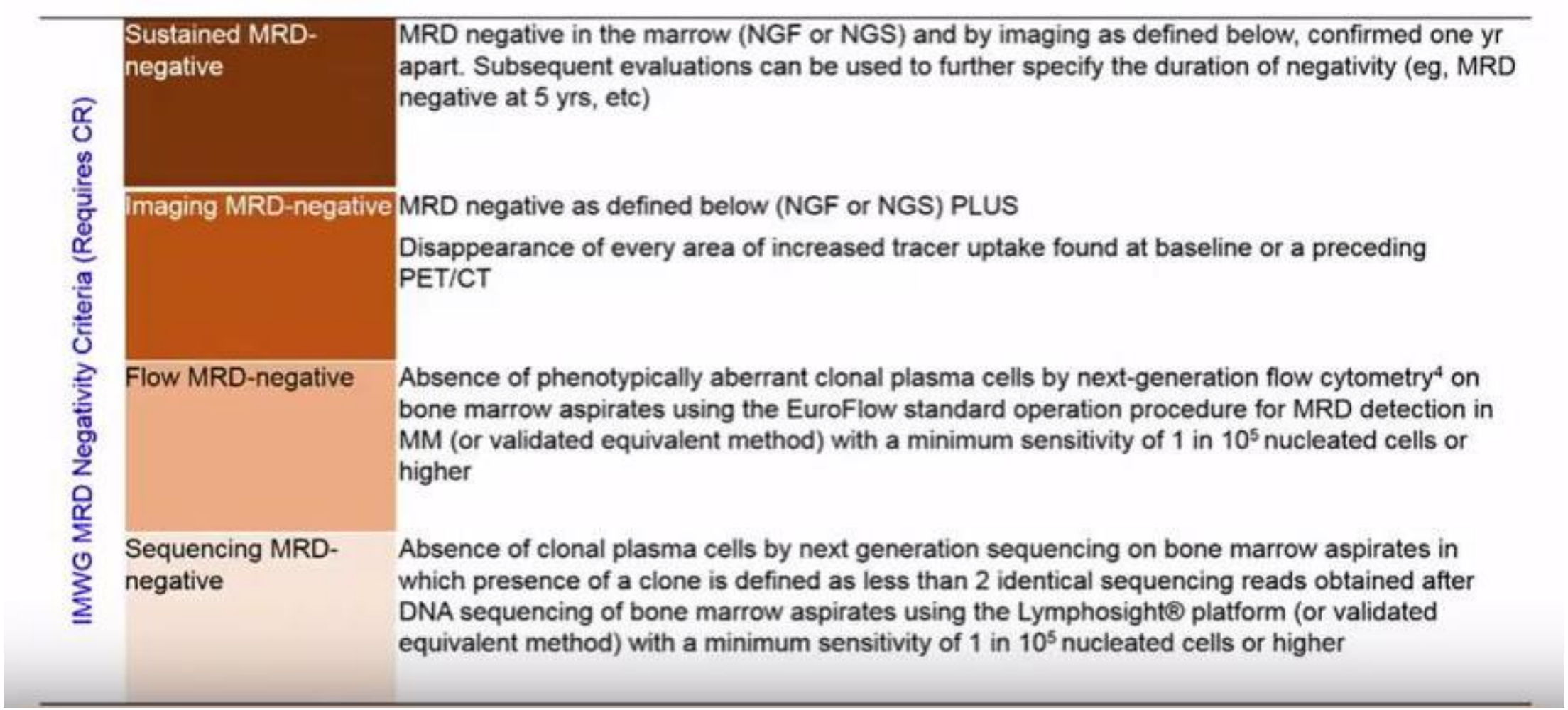
Next generation flow & Next generation sequencing son los métodos de elección para la valoración de la EMR

Criterios de la IMWG para EMR en MM



IMWG MRD Negativity Criteria (Requires CR)	
Sustained MRD-negative	MRD negative in the marrow (NGF or NGS) and by imaging as defined below, confirmed one yr apart. Subsequent evaluations can be used to further specify the duration of negativity (eg, MRD negative at 5 yrs, etc)
Imaging MRD-negative	MRD negative as defined below (NGF or NGS) PLUS Disappearance of every area of increased tracer uptake found at baseline or a preceding PET/CT
Flow MRD-negative	Absence of phenotypically aberrant clonal plasma cells by next-generation flow cytometry[4] on bone marrow aspirates using the EuroFlow standard operation procedure for MRD detection in MM (or validated equivalent method) with a minimum sensitivity of 1 in 10^5 nucleated cells or higher
Sequencing MRD-negative	Absence of clonal plasma cells by next generation sequencing on bone marrow aspirates in which presence of a clone is defined as less than 2 identical sequencing reads obtained after DNA sequencing of bone marrow aspirates using the Lymphosight® platform (or validated equivalent method) with a minimum sensitivity of 1 in 10^5 nucleated cells or higher

Conclusiones

- La citometría de flujo ha demostrado ser relevante en las gamapatías monoclonales en:
  - Identificación de MGUS o MM quiescente de alto riesgo
  - Pronóstico
  - Valoración de Enfermedad Mínima Residual después del tratamiento

gracias
thanks